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Report

Polymorphisms in steroid hormone pathway genes and mammographic density

Christopher A. Haiman[1,5], Susan E. Hankinson[1,2], Immaculata De Vivo[1,2], Chantal Guillemette[3], Naoko Ishibe[4], David J. Hunter[1,2], and Celia Byrne[2]

[1]*Department of Epidemiology, Harvard School of Public Health;* [2]*Channing Laboratory, Department of Medicine, Harvard Medical School and Brigham and Women's Hospital, Boston, MA, USA;* [3]*Oncology and Molecular Endocrinology Research Center, CHUL Research Center (CHUQ), Faculty of Pharmacy, Laval University, Quebec, Canada;* [4]*Genetic Epidemiology Branch, National Cancer Institute, Rockville, MD;* [5]*Department of Preventive Medicine, USC/Norris Comprehensive Cancer Center, Los Angeles, CA, USA*

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Summary

Mammographic density has been linked with exposure to endogenous and exogenous steroid hormones, and increased breast cancer risk. Variation in breast density may be due, in part, to polymorphisms in steroid hormone biosynthesis, metabolism and signaling genes. We conducted cross-sectional analyses within the Nurses' Health Study ($n = 538$), to investigate variation in mammographic breast density, by 10 polymorphisms in eight candidate genes (*CYP17, CYP19, CYP1A1, CYP1B1, COMT, UGT1A1, AR,* and *AIB1*). Breast density was assessed using a computer-assisted technique. We evaluated whether associations between variant alleles of these genes and breast density differed by menopause and postmenopausal hormone (PMH) use. Polymorphisms in *CYP17, CYP19, CYP1B1, COMT, CYP1A1,* or *AR* were not associated consistently with breast density among premenopausal or postmenopausal women. Premenopausal women with the *7/7 UGT1A1* genotype had lower breast density (difference compared to the *6/6* genotype of: −16.5% density; $p = 0.04$). In contrast, postmenopausal women with the *7/7 UGT1A1* genotype had greater breast density compared to those with the *6/6* genotype (+6.2% density; $p = 0.05$); this association was strongest among current PMH users (+13.0% density; $p = 0.03$). In analyses limited to postmenopausal women, breast density was also greater among women carrying short *AIB1* alleles ($\leq$26 glutamine repeats; +4.1% density; $p = 0.04$). Most of the variants in the candidate breast cancer genes evaluated in this study are not strong predictors of breast density. However, our findings of differences in associations for *UGT1A1* and *AIB1* genotypes with breast density by menopausal status needs additional corroboration.

Introduction

Mammographic density is one of the strongest independent predictors of breast cancer risk [1, 2]. Compared to women with no measurable dense breast tissue, women with 75% of their breast being dense have 4–6-fold greater risk of breast cancer [1, 3]. A complex interaction between growth factors and sex steroid hormones is believed to regulate the proliferation of the stromal and epithelial cellular fractions that comprise dense breast tissue observed on a mammogram [4, 5]. Established breast cancer risk factors linked with exposure to endogenous and exogenous steroid hormones, such as age, menopausal status, parity, body mass index and PMH[3] use, have been shown to be associated with variation in breast density [1, 6, 7]. In addition, among premenopausal women, circulating levels of insulin-like growth factor-I (IGF-I) are strongly associated with breast density [8], supporting an association observed between IGF-I levels and premenopausal breast cancer risk [9, 10].

Between-person gene variants may also contribute to the inter-individual variation in breast density observed in the population. It has been suggested that

breast density may be heritable, at least in part, and that multiple genes may be involved [11, 12]. Based on the established role of endogenous steroid hormones and growth factors in the development and maintenance of breast tissue, genes involved in steroid hormone biosynthesis, metabolism and signaling may be important candidates. We hypothesized that polymorphisms in steroid hormone pathway genes may explain variation in breast density.

We evaluated 10 polymorphisms in eight candidate genes in association with breast density among premenopausal and postmenopausal women who were controls in a nested breast cancer case-control study within the Nurses' Health Study (NHS). The genes include those involved in steroidogenesis (*CYP17, CYP19*), the catabolism and elimination of estrogens (*CYP1A1, CYP1B1, COMT, UGT1A1*), and the transcriptional activation of target genes in response to steroid hormones (*AR, AIB1*). We also evaluated whether associations between variant alleles of these genes and breast density differed by menopausal status and PMH use.

Materials and methods

Study population

The NHS was established in 1976 when 121,700 female registered nurses (age, 30–55 years) returned a completed mailed questionnaire. Since that time, biennial mailed questionnaires have been sent to update exposure histories and ascertain changes in medical health status. In 1989–1990, 32,826 participants in the NHS provided a blood sample. Blood collection and sample storage methods have been detailed in a previous publication [13].

The women eligible for this study ($n = 620$) were controls from the nested breast cancer case-control study designed to evaluate genetic and hormonal hypotheses from the NHS subcohort who gave blood [9, 14]. Eligible participants had no history of cancer (except nonmelanoma skin cancer). For each subject, we tried to obtain a mammogram taken as close as possible to the date of blood collection. The study sample for this analysis is comprised of 538 women (87% of eligible) for whom we obtained a usable mammogram.

Mammographic density analysis

To assess mammographic density, the cranial–caudal views of both breasts were digitized at 261 microns/pixel with the Lumysis 85 laser film scanner, which covers a range of 0–4.0 optical density. Details regarding the computer-assisted thresholding software developed at the University of Toronto used to determine the total breast area and the area of the dense mammographic appearance, based on variations in gray scale, have been published previously [15, 16]. In brief, the film screen images are digitized and viewed on a computer screen. For each image, the observer sets the appropriate threshold level that defines the edge of the breast. Next, within this region of interest determined by the edge of the breast threshold, the observer sets a second threshold level delineating the dense area of the image viewed on the screen. The computer calculates the total number of pixels within the entire region of interest and that within the region identified as dense; from these values, the percentage of the breast area that appears dense is estimated. This measure of mammographic breast density has been shown to predict breast cancer risk [3] and is highly reproducible [2]. All readings were made by one reader, and the inter-class correlation between a subset of repeated readings was 0.93. For this study, one breast side (left or right) was randomly selected for determination of breast density.

Genotyping analysis

We genotyped 10 polymorphisms in eight genes (*CYP17, CYP19, COMT*, *CYP1B1*, *UGT1A1*, *CYP1A1*, *AIB1*, and *AR*). All genotyping protocols will be provided upon request from the corresponding author. Fewer postmenopausal women were evaluated in analyses of *CYP1A1* and *CYP1B1* because only one control matched to each breast cancer case was genotyped for polymorphisms in these genes. Polymorphisms evaluated are listed in Table 1.

Exposure data

Age at mammography, weight, height, reproductive history, alcohol intake, menopausal status and use of postmenopausal hormones were ascertained from biennial questionnaires from 1976 until date of mammogram and an additional questionnaire completed at the time of blood sampling. Menopausal status at the time of the mammography was based on responses from the closest biennial questionnaire before the date of the mammogram. Women were considered premenopausal if they reported that their periods had not ceased permanently or having had a hysterectomy with at least one ovary retained and were <49 years of age (nonsmokers) or <47 years (smokers). Women

Table 1. Genetic polymorphisms evaluated in relation to mammographic density

Gene	Encoded product	Polymorphism	Location	Reference
CYP17	Cytochrome P450c17alpha	T27C (*A1*, *A2* alleles)	5′UTR	[17]
CYP19	Aromatase	$(TTTA)_n$ microsatelite,	Intron 4	[18]
		$n = 7$–13, T–C	Exon 10, 3′UTR	[19]
COMT	Catechol-*o*-methyltransferase	G–A (Val158Met)	Exon 4	[20]
CYP1B1	Cytochrome P450 1B1	G–C (Val432Leu)	Exon 3	[21]
UGT1A1	UDP-glucuronosyltransferase 1A1	$(A(TA)_nTAA)$ repeat,	Promoter region	[22]
		$n = 6$ or 7		
CYP1A1	Cytochrome P450 1A1	A–G (Ile462Val, *m2*),	Exon 7,	[23]
		T6235C, *m1*	3′UTR	[24]
AIB1	Amplified in breast-1	CAG + CAA,	Poly-glutamine region	[25]
		glutamines, $n = 19$–34		
AR	Androgen receptor	$(CAG)_n$ repeat,	Exon 1	[26]
		glutamines, $n = 6$–35		

were defined as postmenopausal if they reported that their periods had ceased permanently due to a natural menopause, radiation-induced menopause, bilateral oophorectomy, or surgical menopause with one or more ovaries retained and were >54 years (smokers) or >56 years (nonsmokers). These are the ages at which 90% of the participants in NHS who had a natural menopause were premenopausal or postmenopausal, respectively. Women not included in these two groups were classified as being of uncertain menopausal status. All other covariates were assessed on the basis of the questionnaire preceding mammography; the average time between mammography and the preceding questionnaire was 12 months. This study was approved by the Committee on the use of Human Subjects in Research at Brigham and Women's Hospital.

Statistical analysis

Generalized linear models were used to estimate least-squared mean breast density for each genotype. Indicator variables for multiple genotype combinations were created for genes with >2 alleles (*CYP19, AR,* and *AIB1*). Gene dosage was also evaluated by modeling genotype as an ordinal variable when appropriate. We analyzed both the total area of dense breast tissue, and the percentage of the total breast area that is dense; results were similar for both measures and those for the percentage of the total breast area are presented. We also report the absolute differences in percent breast density between genotypes. In these multivariate models we adjusted for the following predictors of breast density: age (continuous, years), body mass index (BMI) (continuous, kg/m^2), menopausal status (pre-, post-, or uncertain), PMH use status at mammography (current, past or never user), current alcohol consumption at mammography (none, <5, 5–14.9, 15+ g/day), and parity/age at first birth (nulliparous, 1–2 children/age at first birth <25, 1–2 children/age at first birth ≥25, 3+ children/age at first birth <25, 3+ children/age at first birth ≥25). Interactions between genotype and PMH use status were evaluated by including multiplicative interaction terms between genotype (dichotomous if two genotypes; ordinal if three genotypes) and PMH use status (dichotomous, current use v.s. past + never use) in regression models. The Wald statistic p-value was used to assess the statistical significance ($p < 0.05$) of these multiplicative interactions. Data were analyzed with SAS software [27].

Results

There were 94 premenopausal and 392 postmenopausal women, with mean ages of 48.7 (SD, 3.0) and 61.8 (SD, 5.2) years, respectively, at the time of their mammogram. Premenopausal women had a greater mean percentage of breast density than postmenopausal women (37.7% v.s. 21.2%; $p = 0.0001$) (Table 2). Among postmenopausal women, mean breast density was greater among current PMH users compared to past and never PMH users (current users, 26.3%; past users, 18.1%; never users, 18.7%; current

Table 2. Mean percentage of breast density by descriptive characteristics among study subjects ($n = 538$)

Characteristic	$(n)\%$[a]	Mean % density
Menopausal status		
Premenopausal	94(17)	37.7
Postmenopausal	392(73)	21.2
Undetermined	52(10)	
Age (years)		
<50	72(13)	39.3
50–54	82(15)	30.9
55–59	96(18)	20.3
60–64	150(28)	21.5
65–69	101(19)	18.8
>69	37(7)	22.0
Postmenopausal hormone status[b,c]		
Never User	155(40)	18.7
Past User	97(25)	18.1
Current User	140(36)	26.3
BMI (kg/m^2)[c]		
<22	129(24)	35.1
22–24.9	172(32)	29.4
25–29.9	144(27)	19.1
≥30	93(17)	10.0
Alcohol consumption (g/day)[c]		
None	173(34)	27.3
<5	164(32)	21.1
5–14.9	110(22)	25.8
15+	60(12)	26.4
Parity (children)[c]		
0	50(10)	32.6
1–2	147(28)	25.6
3+	330(63)	22.7
Age at first birth (yrs)[c,d]		
<25	256(54)	21.1
25–30	197(41)	26.4
>30	24(5)	27.8

[a] Numbers do not add to 538 due to other categories or missing data.
[b] Among postmenopausal women only.
[c] Age-adjusted mean percent breast density.
[d] Among parous women.

versus past + never PMH users; $p = 0.0001$). As expected, percent breast density was also greater for specific categories of established predictors of mammographic density (Table 2).

Among all women combined, we observed no significant association between the *A2* allele of *CYP17*, or $(TTTA)_n$ repeat or C–T polymorphisms of *CYP19* and breast density (Table 3). Among postmenopausal women, *CYP17 A2* homozygotes had slightly lower breast density (v.s. *A1/A1* genotype; −2.5% density). Although this association appeared limited to current PMH users (−8.3% density), the *p*-value for interaction between *CYP17* genotype and PMH status was 0.64. We observed little evidence that breast density differed by status of the *CYP19* $(TTTA)_n$ repeats previously suggested to be associated with breast cancer risk [28] (v.s. non-carriers of the $(TTTA)_{10}$ or $(TTTA)_{12}$ repeat alleles: −6.6% density for the $(TTTA)_{10}$ allele ($p = 0.81, n = 7$) and +9.1% density for the $(TTTA)_{12}$ allele ($p = 0.56, n = 24$). Among current PMH users, carriers of the *C* allele of *CYP19* had greater density, while among women not currently taking PMH, *T* allele homozygotes had greater density (Table 3).

Among all women combined, we did not observe breast density to differ substantially by *COMT*, *CYP1B1*, or *CYP1A1* genotype status (Table 3). However, we did observe suggestive associations in stratified analyses. Among premenopausal women, compared with non-carriers of the *Met* allele of *COMT*, women with the *Met/Met* genotype had greater breast density (+9.2% density). Among postmenopausal women, those with the *Val/Met* and *Met/Met* genotypes had reduced density (v.s. *Val/Val* genotype: −3.8% density for the *Val/Met* genotype; −2.7% density for the *Met/Met* genotype). We also observed different directions of the association between *COMT* genotype and breast density according to PMH status (*p* interaction, 0.09). Among never and past PMH users, carriers of the *Met* allele had lower density (*p* trend, 0.04). For current PMH users, women with the *Met* allele had greater density.

We did not observe an association between *CYP1B1* genotype and breast density among premenopausal or postmenopausal women, or among non-PMH users. However, we did observe suggestive evidence that current PMH users with the *Val/Val* genotype of *CYP1B1* had higher breast density (v.s. *Leu/Leu* genotype; −7.7% density). We did not observe associations between *CYP1A1* variants (*m1* or *m2*) and breast density, among all women, or by menopausal status (data not shown); due to the low frequency of the variants in *CYP1A1* we had limited power to assess modification of effects by PMH use status.

UGT1A1 genotype was a predictor of mean breast density within menopause status groups (Table 3). Among premenopausal women, women homozygous

Table 3. Associations between polymorphisms in hormone biosynthesis and metabolism genes and percent mammographic density

Genotype[a]	All women		Premenopausal		Postmenopausal		Current PMH user		Never+past PMH use		*p* interaction[e]
	Mean[b] (*n*)	*p*-value	Mean[c] (*n*)	*p*-value	Mean[d] (*n*)	*p*-value	Mean[c] (*n*)	*p*-value	Mean[c] (*n*)	*p*-value	
CYP17											
A2/A2	22.7(76)	0.29	37.6(13)	0.84	18.9(54)	0.34	19.0(18)	0.13	18.8(36)	0.85	
A1/A2	24.3(270)	0.59	36.3(52)	0.60	21.5(198)	0.94	27.2(73)	0.98	18.3(125)	0.98	
A1/A1	25.2(187)	Ref.	39.3(26)	Ref.	21.4(138)	Ref.	27.3(47)	Ref.	18.2(91)	Ref.	0.64
p trend		0.30		0.76		0.47		0.23		0.87	
CYP19 T–C											
CC	25.0(120)	0.95	42.3(20)	0.96	20.0(91)	0.86	25.7(38)	0.24	17.4(53)	0.25	
CT	24.1(268)	0.54	33.2(43)	0.08	22.1(194)	0.40	29.9(62)	0.02	17.8(132)	0.22	
TT	25.2(143)	Ref.	42.6(27)	Ref.	20.4(104)	Ref.	20.6(39)	Ref.	20.6(65)	Ref.	0.07
p trend		0.91		0.78		0.90		0.22		0.23	
COMT											
Met/Met	24.3(149)	0.47	43.1(28)	0.18	20.9(108)	0.25	27.6(45)	0.42	17.4(63)	0.04	
Met/Val	23.8(255)	0.29	35.2(45)	0.83	19.8(184)	0.07	26.2(59)	0.60	16.5(125)	0.006	
Val/Val	25.8(127)	Ref.	33.9(18)	Ref.	23.6(96)	Ref.	24.0(33)	Ref.	22.7(63)	Ref.	0.09
p trend		0.50		0.15		0.27		0.43		0.04	

Table 3. (*continued*)

Genotype[a]	All women		Premenopausal		Postmenopausal		Current PMH user		Never+past PMH use		*p* interaction[e]
	Mean[b](*n*)	*p*-value	Mean[c](*n*)	*p*-value	Mean[d](*n*)	*p*-value	Mean[c](*n*)	*p*-value	Mean[c](*n*)	*p*-value	
CYP1B1											
Leu/Leu	24.1(148)	0.76	37.2(30)	0.70	18.7(101)	0.27	22.1(47)	0.11	15.1(54)	0.78	
Leu/Val	27.7(172)	0.27	38.7(39)	0.15	24.7(118)	0.38	28.0(61)	0.71	20.3(57)	0.28	
Val/Val	24.9(77)	Ref.	34.7(20)	Ref.	22.1(49)	Ref.	29.8(27)	Ref.	16.2(22)	Ref.	0.70
p trend		0.49		0.74		0.11		0.07		0.44	
UGT1A1											
7/7	21.9(51)	0.48	21.8(11)	0.04	25.6(31)	0.05	35.9(12)	0.03	19.6(19)	0.59	
7/6	25.4(231)	0.33	41.6(39)	0.50	21.5(177)	0.22	27.6(59)	0.17	18.1(118)	0.81	
6/6	23.8(242)	Ref.	38.3(39)	Ref.	19.4(175)	Ref.	22.9(65)	Ref.	17.7(110)	Ref.	0.16
p trend		0.99		0.21		0.04		0.02		0.62	

[a] Numbers vary between genes because not all women were successfully genotyped for all polymorphisms.
[b] Adjusted for age, alcohol intake, age at first birth, parity, and, bmi, menopausal and PMH status at mammogram.
[c] Adjusted for age, alcohol intake, age at first birth, parity, and bmi at mammogram.
[d] Adjusted for age, alcohol intake, age at first birth, parity, and, bmi and PMH status at mammogram.
[e] *p*-value for interaction between genotype and PMH status in postmenopausal women.

for the *7* allele had lower breast density (v.s. *6/6* genotype; −16.5% density). The relationship between *UGT1A1* genotype and breast density was in the opposite direction among postmenopausal women, with *7* allele homozygotes having greater density (+6.2% density). This association was strongest among current PMH users, (+13.0% density; p trend, 0.02), the p-value for interaction by PMH use was $p = 0.16$.

We also observed associations between *AIB1* genotype and breast density (Table 4). Among postmenopausal women, carriers of one or more short *AIB1* alleles (≤26 glutamines) had greater breast density (+4.1% density); results were similar in both current and non-current PMH users. We also evaluated the association by duration of current PMH use, as we had previously observed an association between shorter *AIB1* alleles (≤26 glutamines) and increased breast cancer risk among current users of PMH for 5 or more years [29]. In the present study, among the 80 women currently using PMH for 5 or more years, those with shorter *AIB1* alleles did not have greater breast density ($p = 0.99$). However, among women currently taking PMH for less than 5 years, shorter alleles were associated with greater density (+11.3% density, $p = 0.11$). We did not observe breast density to be materially altered by *AR* $(CAG)_n$ repeat length (Table 4). In a previous study, we observed a positive association between *AR* genotype and breast cancer risk limited to women with two long alleles (≥22 CAG repeats) and a family history of breast cancer [30]. In the present study, we did not observe women in this dual category to have substantially greater breast density (v.s. women without a family history of breast cancer and both alleles with <22 CAG repeats; +3.1% density, $p = 0.29$).

Discussion

In this preliminary study of variation in candidate steroid hormone pathway genes in relation to mammographic density, we did not observe strong associations between polymorphisms in *CYP17*, *CYP19*, *COMT*, *CYP1B1*, *CYP1A1*, or *AR* and mammographic density. Our results do suggest however, that variant alleles of *UGT1A1* and *AIB1* may influence breast density. We found the *7* allele of *UGT1A1* to be associated with lower breast density in premenopausal women and greater breast density in postmenopausal women, primarily among current PMH users. We also observed a positive association between shorter *AIB1* repeat alleles and breast density among postmenopausal women.

In the only other cross-sectional study to evaluate genetic polymorphisms in candidate genes in relation to breast density (African–American women, $n = 152$; Caucasian women, $n = 244$) [31], the *A2* allele of *CYP17* was not observed as a strong predictor of breast density. In this prior study, among premenopausal women, the low-activity *Met/Met* genotype of *COMT* was associated with greater breast density among current PMH users (+11.7%, p trend, 0.01). Our findings among Caucasian women are not fully supportive, as we observed little evidence of an association between the *Met/Met* genotype of *COMT* and breast density among current PMH users. In contrast, we observed an inverse association between the *Met/Met* genotype and breast density among never and past PMH users. Although the present study included a substantially larger number of postmenopausal women, differences in the ethnic distribution and the prevalence and duration of PMH between women in these studies may account for the disparate results.

The promoter of *UGT1A1* contains a polymorphic TATA box $A(TA)_nTAA$, with additional $(TA)_n$ repeats demonstrated to reduce expression of the encoded enzyme [32, 33]. This polymorphism may also result in inter-individual differences in the maintenance of steady-state levels of steroids in breast tissue and in the circulation. In our study, the direction of the relationship between *UGT1A1* genotype and breast density varied by menopausal status and was strongest among current PMH users. Based on the direction of the changes in breast density observed among women with different *UGT1A1* genotypes, we would expect premenopausal women with the *7* to have a decreased risk of breast cancer, and postmenopausal women with the *7* allele to have increased risk. In a large nested case-control study in the Nurses' Health Study [34] from whom the current women are a subset, we did not observe *UGT1A1* genotype to substantially influence breast cancer risk among premenopausal or postmenopausal women, or among women currently using PMH. It is not known whether this polymorphism in *UGT1A1* has a direct effect on altering estrogen glucuronidation, or, if inter-individual differences in estrogen glucuronidation influence breast density or breast cancer risk. The *UGT1A1* allele may be associated with breast density, however, given the lack of an association between *UGT1A1* genotype and breast cancer risk, the effect on breast density may be insufficient to modify breast cancer risk via this pathway.

Table 4. Associations between repeat polymorphisms in *AIB1* and *AR* genes and percent mamographic density

Genotype[a]	All women		Premenopausal		Postmenopausal		Current PMH users		Never+past PMH users		*p* interaction[e]
	Mean[b] (*n*)	*p*	Mean[c] (*n*)	*p*	Mean[d] (*n*)	*p*	Mean[c] (*n*)	*p*	Mean[c] (*n*)	*p*	
AIB1											
≤26	26.1(125)	0.27	34.3(25)	0.37	24.3(91)	0.04	31.2(24)	0.17	20.6(67)	0.13	
others	24.1(409)	Ref.	39.0(68)	Ref.	20.2(298)	Ref.	25.3(114)	Ref.	17.4(184)	Ref.	0.53
AR											
both ≥ 22	23.5(116)	0.81	37.4(18)	0.77	20.2(86)	0.83	24.2(34)	0.75	17.2(52)	0.83	
1 ≥ 22	25.3(283)	0.53	37.1(49)	0.68	22.3(205)	0.21	27.2(75)	0.74	19.9(130)	0.14	
0 ≥ 22	24.1(136)	ref.	39.4(26)	Ref.	19.7(99)	Ref.	25.8(30)	Ref.	16.6(69)	Ref.	0.44
p trend		0.86		0.75		0.78		0.74		0.68	

[a] Numbers vary between genes because not all women were successfully genotyped for all polymorphisms.
[b] Adjusted for age, alcohol intake, age at first birth, parity, and, bmi, menopausal and PMH status at mammogram.
[c] Adjusted for age, alcohol intake, age at first birth, parity, and bmi at mammogram.
[d] Adjusted for age, alcohol intake, age at first birth, parity, and, bmi and PMH status at mammogram.
[e] *p*-value for interaction between genotype and PMH status in postmenopausal women.

We also observed suggestive evidence that shorter alleles (≤26 glutamines) of the *AIB1* gene may predict increased breast density among postmenopausal women. This association was similar among both current, and never or past PMH users. AIB1 is a steroid receptor coactivator protein that interacts with estrogen receptor-α in a ligand-dependent manner resulting in increased estrogen-dependent transcription [35]. Enhanced transcription of estrogen responsive genes may increase breast cell proliferation and lead to dense breast tissue. We previously observed a suggestive association between shorter *AIB1* alleles and breast cancer risk only among current PMH users who had used hormones for 5 or more years (OR, 1.98; CI, 0.96–4.07) [29]. In the present study, percent breast density levels were similar among carriers and noncarriers of shorter alleles (≤26 glutamines) among women currently using PMH for 5 or more years, however, we had limited power to investigate this relationship due to the small number of women currently using PMH for 5 or more years ($n = 80$).

The genetic polymorphisms in this study were evaluated based on a biological rationale of potential relevance to endogenous steroid hormones production, elimination and action, and breast cancer risk. Given this is one of the first studies of percent breast density and genetic polymorphisms, we evaluated a number of associations using tests of statistical differences as a method to identify potentially meaningful differences in mean breast density across polymorphisms. We recognize that some of the differences we noted may not represent true differences (false-positives), particularly given the large number of comparisons made, and hope that others will soon evaluate these associations in other populations for comparison. False-negative associations may also exist, as this study has limited power to comprehensively assess associations among premenopausal women, and by PMH use status. Larger studies will be required to confirm these observations.

Polymorphisms in these candidate genes have been hypothesized to alter a woman's hormonal milieu, or alter the level of potential mammary carcinogens or hormone-responsive gene transactivation in breast tissue, and thus, increase susceptibility to breast cancer. A 1% increase in breast density has been estimated to increase breast cancer risk by 1.4% [1]. We would therefore predict that if these variant alleles increase breast cancer risk via altering breast density, then they would be associated with only modest changes in breast cancer risk. Specific alleles of these genes that were observed to influence breast density in this study have not been consistently associated with breast cancer risk in previous studies. In addition, polymorphisms we have observed to be associated with increased breast cancer risk, such as the *10* and *12 TTTA* repeat alleles of *CYP19* [28] were not associated with breast density in the present study. Additional work is needed to clarify the role of polymorphisms in candidate breast cancer susceptibility genes as predictive markers of mammographic density.

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Address for offprints and correspondence: Celia Byrne, Channing Laboratory, 181 Longwood Avenue, Boston Massachusetts, USA